Endemic Kaposi's Sarcoma in Human Immunodeficiency Virus Type 1 – Seronegative Persons: Demonstration of Retrovirus-Like Particles in Cutaneous Lesions

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In 1984, Greek physicians reported on the clustering of cases of Kaposi's sarcoma (KS) on the Peloponnesus peninsula. To gain more insight into its pathogenesis, we studied the sero-epidemiologic and clinicopathologic characteristics of 12 Greek KS patients (eight male/four female) five of whom were residents of an endemic area on the Peloponnesus. These patients were in good general health with ages ranging from 48 to 80 years, had no clinical signs of immunodeficiency, and combined the features of both classic and epidemic KS in that they displayed not only involvement of acral areas but also widespread mucocutaneous lesions. Routine laboratory data were within normal limits; no patient had HTLV-1 and HIV-1/2 antibodies, but all patients had antibodies to several herpesviruses. The histopathology was characteristic of KS with the peculiar feature of a dense infiltrate composed predominantly of CD4+ T lymphocytes. Immunoenzymatic/morphologic studies of the KS cells were consistent with their origin from lymphatic endothelium. Outstanding ultrastructural findings were tubuloreticular structures and cylindrical confronting cisternae, structures that are indicative of an ongoing viral infection. Indeed, extensive electronmicroscopic studies resulted in the detection of retrovirus-like particles in close association to KS cells in five of 12 patients. This in situ observation opens the possibility that this retro-virus contributes to KS development. *J Invest Dermatol 95:371–381, 1990*

Clinicopathologically, four distinctive forms of Kaposi's sarcoma (KS) are recognized: (a) the classical variant, which frequently occurs among elderly men of Jewish Ashkenazi ancestry and usually runs a rather benign course over years [1,2]; (b) the endemic African form, which involves children, adolescents, and adults with a high frequency of extracutaneous manifestations and a more aggressive course [3,4]; (c) KS seen in organ transplant recipients undergoing immunosuppressive therapy [5–7]; (d) epidemic KS representing the most important opportunistic neoplasm occurring in human immunodeficiency virus type 1 (HIV-1)–infected individuals [8–12]. The pathogenic mechanisms leading to the histopathologically uniform but clinically diverse KS variants are still unclear. Although immunosuppression apparently contributes to the devel-

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Abbreviations:
AIDS: acquired immunodeficiency syndrome
CCC: cylindrical confronting cisternae
CMV: cytomegalovirus
EA: EBV early antigen
EBNA: EBV nuclear antigen
EBV: Epstein-Barr virus
ELISA: enzyme-linked immunosorbent assay
HHV-6: human herpes virus type 6
HIV-1,2: human immunodeficiency virus types 1 or 2
HSV: herpes simplex virus
HTLV-1: human T-lymphotropic virus 1
IF: immunofluorescence
Ig: immunoglobulin(s)
KS: Kaposi's sarcoma
MoAb: monoclonal antibody(ies)
PBS: phosphate-buffered saline
RT: room temperature
TPHA: treponema pallidum hemagglutination test
TRS: tubulo-reticular structures
VCA: EBV capsid antigen
VDRL: venereal disease research laboratory test
vWF: von Willebrand factor
VZV: varicella zoster virus

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Figure 1. Clinical features of mucocutaneous KS lesions in Greek patients. *a*, *b*, involvement of the facial skin by multiple KS lesions in patch and plaque stage *(a)*; prominent, plaque-like, and nodular lesions on the left ear *(b)*. *c–e*, disseminated, streaky, and coin-sized lesions on the extremities. *f*, KS lesions in all stages involving both feet. *g*, *h*, mucosal KS lesions involving the glans penis *(g)* and the hard palate *(h)*.

Table I. List of First-Step Antibody Reagents

Reagents[a]	Working Dilution	Reactivity on Normal Human Tissue	Source[b]	Relevant References
PAL-E	1:500	Endothelial cells of mature blood vessels; dermo-epidermal junction	Serotec	[20,21]
EN-4	1:500	Endothelial cells of mature blood and lymphatic vessels	Serotec	[21,22]
Anti-von Willebrand Factor	1:500	Endothelial cells of mature blood vessels	Dakopatts	[23,24]
OKM-5 (anti-CD36)	1:300	Endothelial cells of mature blood vessels; subset of tissue macrophages	Ortho	[24,25]
Anti-laminin	1:500	Basement membrane zone protein	Serotec	[26,27]
Anti-collagen type IV	1:2000	Basement membrane zone protein	Serotec	[26,27]
Anti-desmin	1:100	Intermediate-sized filaments of smooth muscle cells	Dakopatts	[28]
Anti-vimentin	1:50	Intermediate-sized filaments of certain mesenchymal (e.g., endothelial cells, fibroblasts, macrophages) and neuroectodermal (e.g., melanocytes, Schwann cells) cells	Dakopatts	[29]
Anti-leucocyte (HLe-1) (anti-CD45)	1:200	All human leucocytes	Becton-Dickinson	[30]
Anti-Leu-4 (anti-CD3)	1:600	T-cell antigen associated with T-cell receptor	Becton-Dickinson	[31]
Anti-Leu-3a (anti-CD4)	1:200	Human T helper/inducer cell antigen	Becton-Dickinson	[31]
Anti-Leu-2a (anti-CD8)	1:150	Human T cytotoxic/suppressor cell antigen	Becton-Dickinson	[31]
Anti-Leu-M3 (anti-CD14)	1:100	A human monocyte/macrophage antigen	Becton-Dickinson	[32]
B1 (anti-CD20)	1:200	A human B lymphocyte antigen	Coulter	[33]
Anti-Factor XIIIa	1:20,000	Factor XIIIa expressed on mononuclear phagocytes including dermal dendrocytes	Calbiochem	[34]

[a] All reagents were monoclonal antibodies (MoAb) with the exception of the polyclonal rabbit-anti human Factor XIIIa antiserum.
[b] Serotec, Oxford, England, UK; Dakopatts, Copenhagen, DK; Ortho Diagnostic Systems, Raritan, NY, USA; Becton-Dickinson Immunocytometric Systems, Mountain View, CA, USA; Coulter Immunology, Hialeah, FL, USA; Calbiochem Corporation, San Diego, CA, USA.

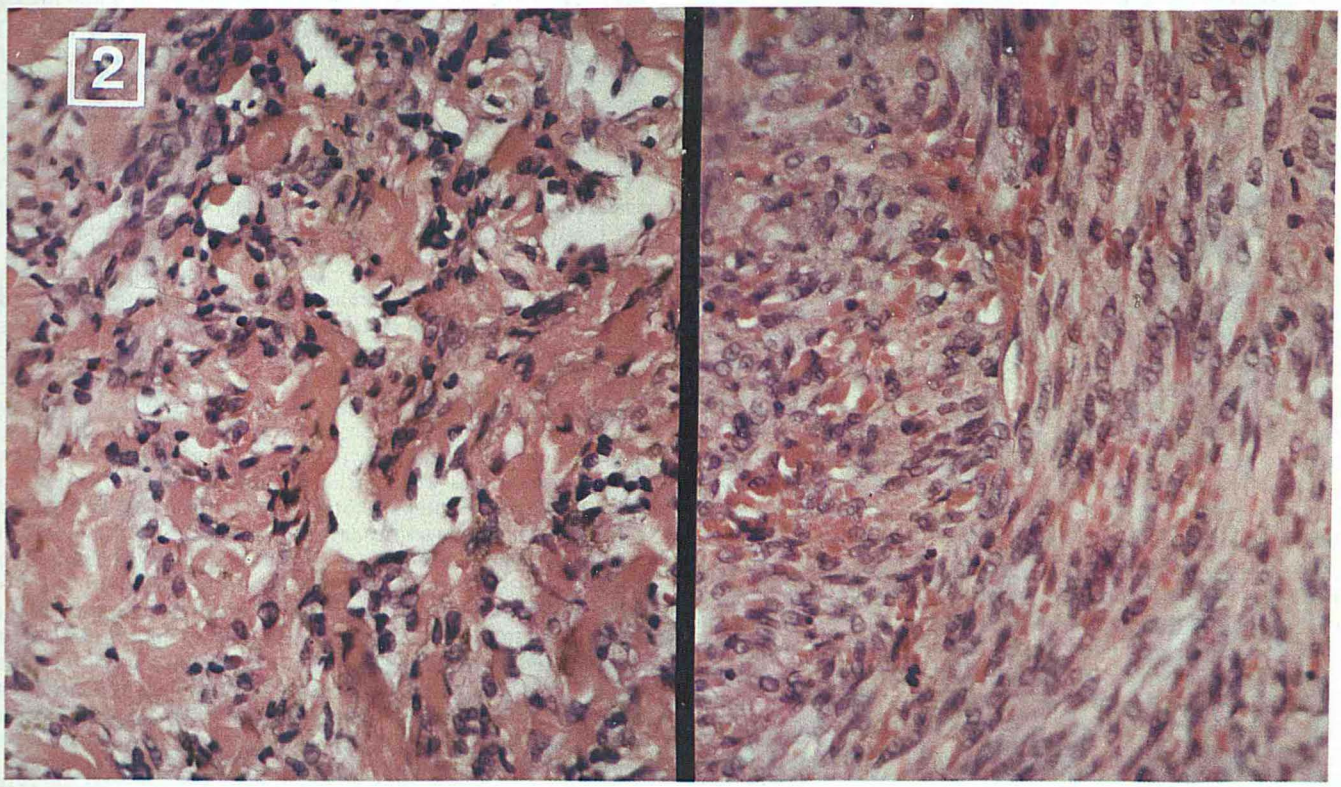

Figure 2. Histopathology of KS lesions of Greek patients. *a*, angiomatous KS: note the irregular size and shape of vascular lumina, outlined by a monolayer endothelium. *b*, sarcomatous KS: spindle cells forming solid tumor fascicles; red blood cell extravasation between the tumor cells.

Table II. Mucocutaneous Localization of KS Lesions in Greek Patients[a]

Patient	Feet	Thighs	Hands	Arms	Head/Face	Trunk	Oral/Genital Mucosa	Gastrointestinal Tract		
								Esophagus	Stomach	Duodenum
1	+	+	+	+	+	−	−/−	−	++	−
2	++	+	++	−	−	−	−/−	n.d.	n.d.	n.d.
3	++	++	++	+	++	−	+/+	−	++	−
4	++	+	−	−	−	−	−/−	−	++	−
5	+	+	++	+	−	−	−/−	−	++	−
6	+	−	++	−	+	+	+/−	−	++	+
7	++	−	+	+	−	−	+/+	−	−	−
8	++	−	+	+	−	−	−/−	−	+	+
9	−	+	−	−	+	−	−/−	+	+	+
10	−	−	−	++	−	−	+/+	−	++	−
11	++	++	−	−	−	−	−/−	−	++	++
12	+	++	+	++	−	−	−/−	n.d.	n.d.	n.d.

[a] Grade of involvement by KS: +, moderate; ++, severe; −, not involved; n.d., not explored by endoscopy.

opment of KS in transplant recipients [7], certain epidemiologic aspects of KS [3] as well as the lack of correlation between severity of immunosuppression and frequency of KS in some HIV-1–infected persons [2] essentially exclude the possibility that immunosuppression is the only factor that triggers the events leading to this proliferative disorder.

In 1984, Kaloterakis reported a clustering of KS among the inhabitants of distinct areas on the Southern Peloponnesus peninsula in Greece [13]. In 1988, an international study group was formed in order to conduct more extensive epidemiologic and clinicopathologic investigations on this cluster of patients and, by doing so, to contribute to the elucidation of pathomechanisms operative in KS.

PATIENTS AND METHODS

Epidemiology Reportedly, the overall incidence of KS in Greece is $3.5/10^6$ people/year with an endemic focus on the Peloponnesus (7.7 cases$/10^6$ people/year) (Hatzakis et al, in preparation). Twelve KS patients, eight male and four female, consecutively admitted to the Department of Dermatology, Athens University Medical School, were selected for a thorough medical examination. Their ages ranged from 48 to 80 years (mean, 72.6 years) with an average disease duration of 2.5 years (range, 1–5 years). Five patients were born on the Peloponnesus (patients 3, 5, 6, 7, and 12), five were born in other areas of Southern Greece, and two others were born in Turkey and migrated to Greece during their adolescence. All pursued different professional activities but most of them were still living as farmers sometimes under poor sociohygienic conditions. To the best of the patients' knowledge, none of their closer relatives had overt signs of KS. All patients studied were heterosexuals with no history of sexually transmitted diseases. None of them had ever experienced any severe infectious disease nor had anyone received blood transfusions, therapy with blood products, or any immunosuppressive treatment.

Clinical and Routine Laboratory Evaluation In addition to a detailed physical evaluation, patients were subjected to various radiologic, sonographic, and endoscopic procedures. Routine investigations included blood sedimentation rate, complete and differential blood cell counts, serum electrolytes, renal and liver function tests, and syphilis serology (VDRL, TPHA tests).

Virus Serology Cytomegalovirus (CMV), herpes simplex virus (HSV), and varicella zoster virus (VZV) -specific immunoglobulin (Ig)M and IgG antibodies were determined by solid-phase enzyme-linked immunosorbent assays (ELISA) according to established procedures [14,15]. Antibodies against the Epstein-Barr virus (EBV) capsid antigen (VCA), EBV early antigens (EA) and EBV nuclear antigens (EBNA) were determined by indirect immunofluorescence (IF) assays [16,17]. The test for human herpes virus type 6 (HHV-6)-specific IgG antibodies was carried out using the HHV-6 kit from Pan-Data-Systems, Inc. (Rockville, MD).

Seroreactivity testing against human retroviruses was performed in two separate laboratories and included the screening for serum antibodies against (a) HIV-1 by commercial ELISA (Organon Teknika, Turnhout, Belgium) and Western blot (Dupont-Comp. Med. Prod., Wilmington, DE) assays; and against (b) HIV-2 and (c) human T-lymphotropic virus 1 (HTLV-1) by radioimmunoprecipitation assays using appropriate virus-infected cell lines [18,19].

Immunomorphologic Studies on Biopsies from KS Skin Lesions One to three biopsies of KS lesions in different stages of development were taken from each patient from different body regions under local anesthesia with 2% mepivacain; each biopsy was divided into three parts: one part was immediately fixed in 1.96% paraformaldehyde, pH 7.4, and further processed for histopathological examination; a second portion of the specimen was fixed in a mixture of 2% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.4, and then further processed for transmission electronmicroscopic analysis.

For immunohistologic studies, the third part of the biopsy specimen was snap-frozen in Tissue-Tek OCT compound (Miles Scientific, Naperville, IL) using isopentane pre-cooled in liquid nitrogen and then stored at −70°C until further use. Four-micrometer cryostat serial sections were mounted onto gelatine-coated slides, air-dried, and fixed in acetone for 10 min at 4°C. For blocking of Fc-Ig receptors, sections were preincubated for 20–30 min at room temperature (RT) in phosphate-buffered saline (PBS, pH 7.4) containing 20% heat-inactivated sheep serum (GIBCO, Grand Island, NY). For morphologic orientation, the first cryostat section of each tissue block was stained with hematoxylin and eosin.

First-step antibodies used are listed in Table I [20–34].

Immunoenzymatic Staining For single-color staining, the three-step avidin-biotin immunoperoxidase technique was used as described elsewhere [35]. Briefly, sections were incubated with the first-step antibodies (working dilution, see Table I) for 12–16 h at 4°C. Thereafter, the slides were thoroughly washed in PBS, and then reacted with either an appropriately diluted biotin-conjugated sheep anti-mouse Ig (Amersham, Amersham, UK) or a biotin-conjugated donkey anti-rabbit Ig (Amersham) for 1 h at RT. After three PBS washes, slides were further incubated with the streptavidin-biotin peroxidase complex (Strept ABComplex-HRP, Dakopatts; Copenhagen, Denmark) as described in the data sheet. Bound immunoreactants were visualized by using the diaminobenzidine reaction.

For double-labeling purposes, the incubation chain was extended by consecutively exposing the sections to appropriate murine MoAb, biotin-labeled sheep anti-mouse Ig and, finally, avidin-biotin alkaline phosphatase complex (ABComplex-AP, Dakopatts). This enzyme was then visualized with naphthol AS-MX phosphate/Fast Red [35].

Controls Cryostat sections of skin (healthy volunteers) and lymph node specimens (autopsy material) were used to define the optimal working dilutions of the respective antibodies.

Negative controls included (a) omission of either the first or one

Table III. Immunophenotype of KS Cell[a]

MoAb	Normal Human Skin		KS Lesions	
	Endothelium of Mature Blood Vessels/Lymphatic		Endothelium of Clefts/Slits	Spindle Cells
PAL-E	++	−	−	−
EN-4	++	++	+	+
Anti-von Willebrand factor	+	−	−	−
OKM-5 (anti-CD36)	+	−	−/(+)	−/(+)
Anti-laminin	++	+	+	+
Anti-collagen type-4	++	+	+	+
Anti-desmin	−	−	−	−
Anti-vimentin	++	++	++	++

[a] −, negative; (+), occasionally weakly positive; +, weakly positive; ++, strongly positive.

of the subsequent antibody reagents; (b) substitution of the primary antibody either with normal mouse or rabbit serum; (c) substitution of the first antibody with an irrelevant antibody of the same isotype. Consistently, negative results were obtained.

RESULTS

Clinical Features Each of the 12 patients displayed multiple KS lesions in all stages of development (Fig 1). The lesions were most pronounced on the distal portions of the lower and upper extremities but also occurred, at varying frequency, on the proximal parts of the extremities, head, neck, and face. Characteristically, the lesions were predominantly located on acral areas and were usually distributed in a symmetrical fashion (Fig 1, Table II). In addition to the cutaneous lesions, mucosal involvement was observed in 10 patients. Four patients exhibited orogenital KS patches; endoscopic evaluation of the gastrointestinal tract revealed esophageal, gastric, and/or duodenal KS lesions in nine of 10 patients examined (Table II). In one third of the patients, indolent palpable lymph nodes (diameter approximately 2 cm) were noted in the inguinal (patients 3, 4, and 6) and cervical (patients 1 and 6) regions. Unfortunately, lymph node biopsies for histologic examination were not feasible. With the exception of patient 4, who had experienced a weight loss of 10 kg during the last 4 months prior to examination, the patients were in good general health and were not incapacitated by the KS lesions. None of the patients offered a history of overt melena, and radiologic and/or sonographic studies of thorax and abdomen did not reveal any pathologic process.

Laboratory Data The routine laboratory data (blood sedimentation rate, total and differential blood cell count, electrolytes, liver and renal function tests) were essentially within normal limits. Syphilis serology tests were negative in all patients.

Antibodies to HTLV-1, HIV-1, and HIV-2 were not detectable in any of the patients' sera by either ELISA, Western blot, or radioimmunoprecipitation assays. Each patient, however, exhibited detectable IgG antibody titers to HSV, VZV, CMV, HHV-6, and EBV. In the latter instance, the antibody spectrum included IgA antibodies against EBV-VCA and antibodies against EBNA and EBV-EA.

Histopathology The histopathologic examination of biopsy specimens revealed the typical picture of the various stages of KS. The early patch-like lesions predominantly consisted of increased numbers of irregularly sized, jagged-shaped, thin-walled clefts and slits, dissecting the pre-existing dermal collagen bundles (Fig 2*a*). Erythrocytes were frequently detected within these clefts but were occasionally also extravasated in the spongy network of collagen fibers. Elongated cells lining these clefts did not show any signs of atypia and were considered to be endothelial cells. In addition to the changes already seen in the patch stage, the advanced plaque stage showed some vascular neoproliferation consisting of normal-appearing venules and arterioles; a distinct sign of the plaque lesions were solid cords composed of spindle cells arranged in an indian file pattern. This biphasic tumor morphology, exhibiting both angiomatous and solid tumor pattern, changed to a clearcut sarcomatous pattern in the progressed nodular stage. These lesions consisted almost exclusively of spindle cells that were arranged in bundles and interlacing fascicles (Fig 2*b*). Some of the tumors were relatively sharply demarcated, exhibiting a pseudocapsule at the periphery; others were characterized by an invasive growth throughout the entire reticular dermis. In high magnification only, we observed numerous erythrocytes between the individual spindle cells as well as siderophages that were predominantly found at the periphery of the tumors. Signs of pronounced cellular atypia or an increased mitotic rate were rarely seen in any stages of development. A hallmark of all lesions was the admixture of a moderate to dense inflammatory infiltrate, consisting of lymphocytes, histiocytes, and plasma cells (see below).

Immunohistochemistry

Phenotypic Analysis of the Vascular Component of KS Lesions In biopsies of normal human skin from healthy volunteers, we observed that MoAb EN-4 and anti-factor VIII-related antigen (anti-von Willebrand Factor (vWF)) displayed selective reactivity with endothelial cells. Clearcut endothelial cell reactivity was also seen with MoAb PAL-E and OKM-5, but these reagents also reacted with other structures/cells (Table III). In agreement with previous observations, we found (a) that MoAb EN-4 reacted with both lymphatic and blood vessel endothelium; (b) that PAL-E, anti-vWF, and OKM-5 reacted with blood vessel endothelial cells only; and (c) that all endothelial cells expressed vimentin, but failed to react with anti-desmin antibodies (Table III) [21-29].

In all specimens of patch/plaque stage KS lesions, cells lining the typical clefts and slits were uniformly EN-4-positive, but virtually failed to display PAL-E, OKM-5, and anti-vWF reactivity (Table III). At their abluminal side, these cells exhibited distinct anti-laminin and anti-type IV collagen reactivity indicative of the presence of a basal lamina. Thus, the phenotypic profile of these cells is indistinguishable from that of lymphatic endothelial cells in normal human skin (Table III).

In late plaque and tumor-stage KS lesions, spindle cells forming solid tumor nests and bundles were again EN-4$^+$, PAL-E$^-$, and anti-vWF$^-$ and only occasionally displayed weak OKM-5 reactivity (Table III, Fig 3). Remarkably, these regions showed a weak but distinct anti-laminin- and anti-collagen type IV staining. Although this immunolabel could not be ascribed to defined structures, it clearly coincided with tumorous areas and did not exceed their outer contours. Similar to the situation in normal human skin, anti-collagen type IV and anti-laminin staining outside the proliferating spindle cells were confined to basement membranes of both epithelial-dermal junctions and blood vessels. In contrast to the cells lining the pathognomonic vascular channels mentioned above, endothelial cells lining these blood vessels showed strong PAL-E, EN-4, anti-vWF, and moderate OKM-5 immunolabeling (Table III, Fig 3). Finally, it should be emphasized that the spindle cells as well as the endothelial cells were uniformly of the vimentin rather than of the desmin type.

Phenotypic Analysis of Inflammatory Cells Within KS Lesions Immunolabeling of serial sections revealed that EN-4$^+$ cells were lack-

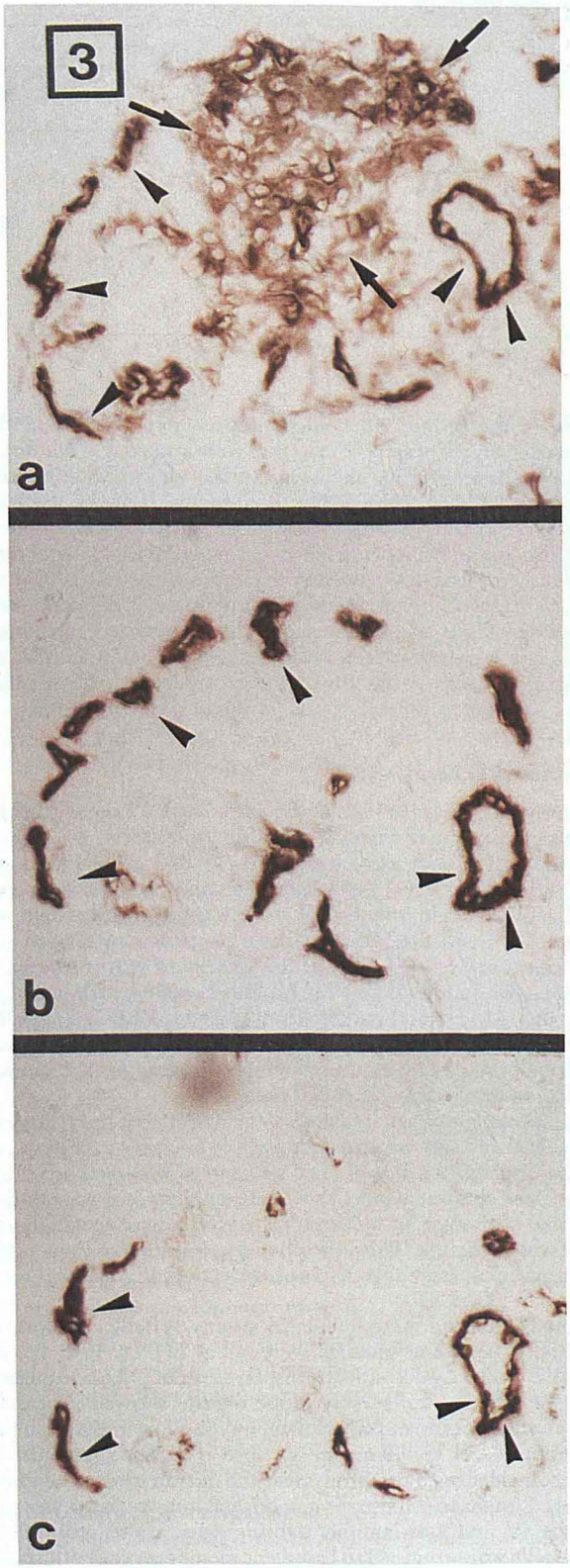

Figure 3. Immunophenotypic analysis of a solid KS lesion on serial-cryostat sections. *a*, mature blood vessels *(arrowheads)* as well as spindle cells *(arrows)* are labeled with MoAb EN-4. *b*, MoAb PAL-E stains endothelial cells of mature blood vessels *(arrowheads)* but fails to react with spindle cells. *c*, labeling with anti-vWF MoAb is restricted to mature blood vessels *(arrowheads)*; the spindle cells are negative.

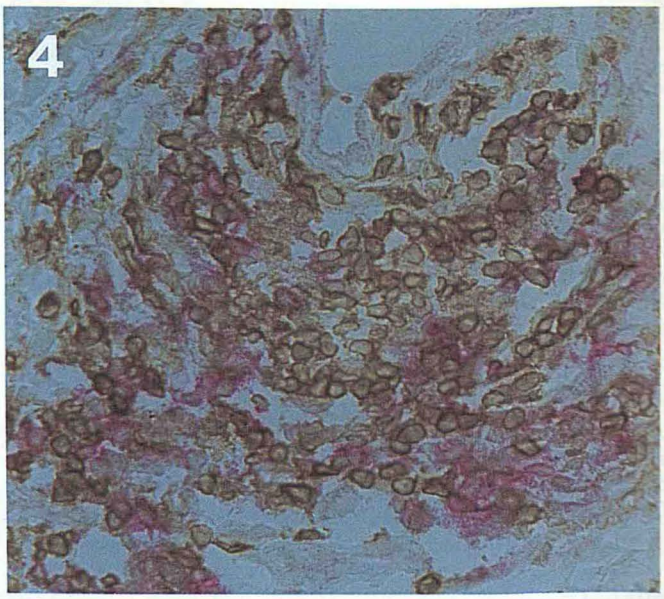

Figure 4. Immunophenotypic analysis of mononuclear cells infiltrating KS lesions. $CD4^+$ cells (brownish reaction product) greatly outnumber $CD8^+$ lymphocytes (red membrane staining.).

ing the common leukocyte antigen CD45. However, considerable numbers of $CD45^+$ cells were seen around patch-stage vascular slits as well as between and in the immediate vicinity of spindle cell cords, nests, and bundles. Most of these cells represented $CD3^+$ lymphocytes which, by appropriate serial sectioning, were predominantly of the CD4 subtype (Fig 4). To a lesser extent, we also identified CD14-bearing macrophages and anti-factor XIIIa-reactive stellate and elongated cells [34]. $CD20^+$ B cells were barely detectable.

Electronmicroscopy

Cell Morphology The irregularly shaped clefts and slit-like spaces of patch and plaque-stage KS lesions were outlined by a monolayer of cells that appeared rather thin with elongated processes, sometimes protruding into the surrounding collagen tissue. The cells formed intercellular connections (tight junctions, desmosome-like structures, overlapping and interdigitating junctions), exhibited large numbers of pinocytotic vesicles and multivesicular bodies but were consistently devoid of Weibel-Palade bodies. At the abluminal side, the cells were inconsistently outlined by a basal lamina but pericytes were not observed (Fig 5*a*). Thus, these cells greatly resemble lymphatic endothelial cells. Most noteworthy was the additional finding of tubulo-reticular structures (TRS, Fig 5*a*), erythrophagocytosis, and membrane ghosts within their cytoplasm.

Spindle cells comprising fascicles displayed similar morphologic features including the presence of intercellular junctions, pinocytotic vesicles, multivesicular bodies, and TRS, but, in addition, also occasionally contained cylindrical confronting cisternae (CCC) (Fig 5*b*). A careful screening (more than 3000 tumor cells examined) for the presence of Weibel-Palade bodies yielded negative results with the exception of two spindle cells each of which contained one single Weibel-Palade body. Most of the spindle cells were in tight contact with each other and, thus, no longer associated with vascular lumina and not surrounded by a basal lamina. Occasionally, however, widened channel-like spaces were observed between neighboring spindle cells and in these instances basal lamina formation was observed at the "abluminal" side (Fig 5*c*).

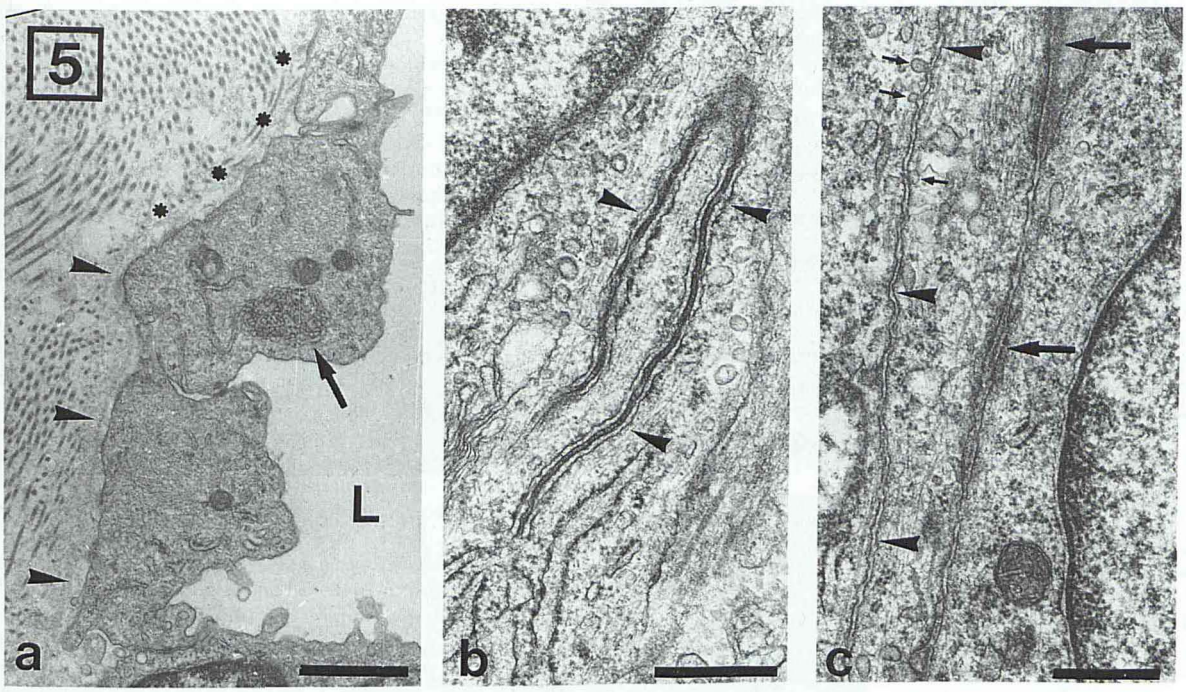

Figure 5. Ultrastructural characteristics of KS cells. *a*, TRS *(arrow)* within a cell forming the endothelium of a slit-like lumen. Note the basal lamina at the abluminal side *(arrowheads)* and its fragmentation *(asterisks)*. No Weibel-Palade bodies can be observed. L, lumen; magnification ×13,000; *bar*, 1 μm.*b*, CCC *(arrowheads)* observed in a spindle cell of a KS lesion; magnification ×30,000; *bar*, 500 nm. *c*, spindle cells form abortive vessel-like channels *(arrowheads)* with multiple pinocytotic vesicles *(small arrows)*; at the "abluminal side" remnants of a basal lamina can be discerned *(bold arrows)*; magnification ×24,000; *bars*, 500 nm.

As opposed to these spindle cells, morphologically normal-appearing blood vessels were lined by cells displaying the typical features of blood vessel endothelial cells, i.e., prominent nuclei and the almost regular presence of Weibel-Palade bodies. These blood vessels were surrounded by a continuous, often multilayered basement membrane and adjoining pericytes.

Retrovirus-Like Particles Extensive electronmicroscopic tissue screening revealed the presence of virus-like particles in biopsy specimens of KS lesions from five patients (patients 3, 5, 8, 11, and 12). These round/globular structures measured 90–130 nm in overall diameter. Some of these particles consisted of a ring-shaped, electron-dense nucleoid (60–70 nm in diameter) and a more electron-lucent center. The ring-shaped nucleoid was surrounded by a slightly electron-transparent intermediate layer that was enveloped by a peripheral unit membrane (Fig 6*a*). Multiple surface knobs with a length of 7–10 nm protruded from this membrane (Fig 6*a*). There existed other particles of similar size that contained a condensed nucleoid of 40–50 nm in diameter (Fig 6*b–d*). This electron-dense core was located either centrally or slightly eccentrically, was surrounded by an electron-lucent layer of 25–30 nm thickness, and was enveloped by a peripheral unit membrane (Fig 6*b–d*). On the surface of this membrane, we occasionally detected truncated, but never full-sized, knobs (Fig 6*b–d*). In essence, the former particles correspond in size and shape to immature retroviral particles and the latter are morphologically indistinguishable from mature oncovirinae [36–38]. These particles were only detected in close vicinity to or, occasionally, even on the surface of tumor cells (Fig 6*b–d*). In one instance, we detected a mature retroviral particle within a coated pit of a spindle cell (Fig 6*e*) indicative of its subsequent uptake. In keeping with this assumption was our additional finding of a similar retroviral particle within a cytoplasmic vacuole of an endothelial cell (Fig 6*f*). Finally, and most importantly, we observed a particle exhibiting the above-described morphologic features budding from the membrane of a cytoplasmic vacuole of a tumor cell (Fig 6*g*).

DISCUSSION

In this report, we present data on a newly recognized endemic occurrence of KS in a defined Mediterranean population. Epidemiologically, this Greek KS variant resembles the endemic KS form occurring in central Africa that was first documented during the 1950s and 1960s [3,4]. The incidence of eight new cases per year per million Peloponnesian inhabitants refers to the entire peninsula. If one focuses on the geographically more restricted regions of the Southern Peloponnesus (Laconia, Messinia), the incidence is 3 to 4 times higher, and thus approximates the rate of occurrence of the African variant [3,4]. It should also be emphasized that the proportion of affected women is exceedingly high when compared to classical and epidemic KS forms [1,2].

The patients in this study presented a combination of the characteristic clinical features of the different KS variants. Although KS lesions in several stages of development on the feet and hands are representative of classic KS and the "nodular"-type of the African KS, widespread, coin-sized, and streaky lesions on the arms and legs are more commonly seen in patients suffering from the acquired immunodeficiency syndrome (AIDS) [1,2,12]. Moreover, the extensive involvement of the facial skin and head, of the oral and genital mucosa and, more importantly, of the gastrointestinal tract is uncommon in the classic, more common in the African, but a typical feature of the AIDS-associated KS [1,2,12].

Confirming earlier histopathologic reports [4,6,12,39], the initial patch-like lesions consisted of increased numbers of bizarre, endothelium-lined clefts and slits which were accompanied by solid tumor cords consisting of spindle cells in the plaque stage and acquiring a sarcomatous appearance in the nodular stage. In accordance with previous observations, we found that cells lining cleft-like

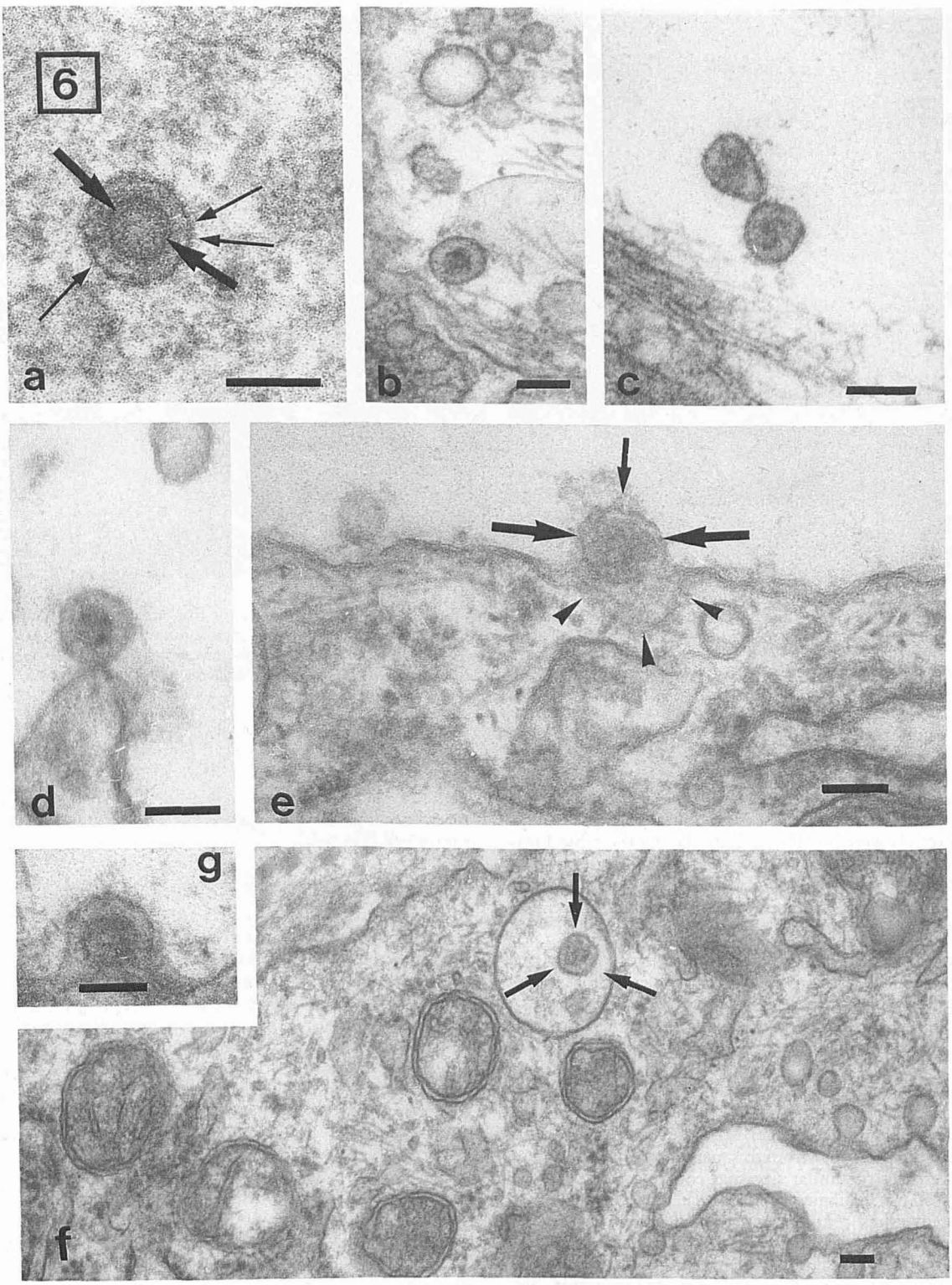

Figure 6. Identification of retroviral particles in KS lesions. *a*, immature extracellular retrovirus. Note the 7–10-nm long spikes/knobs at the enveloping membrane *(thin arrows)* and the ring-shaped nucleoid *(bold arrows)*; magnification ×140,000. *b–d*: several oncovirinae-like structures are found extracellularly, in close vicinity to the cytoplasmic membrane of KS cells. Note the roundish, centrally or slightly eccentrically located nucleoid; magnification ×80,000 *(b)*, ×100,000 *(c)*, ×125,000 *(d)*. *e*, demonstration of a virus-like particle *(large arrows)* in close vicinity to a coated pit *(arrowheads)*. Note the tail-like structure protruding from the envelope of the virus *(small arrow)*; magnification ×100,000. *f*, virus-like particle *(arrows)* within a cytoplasmic vacuole of a KS cell. (L, lumen, magnification ×52,000; *g*, budding of an immature virion from the membrane of a cytoplasmic vacuole of a KS cell; magnification ×100,000. *Bars*, 100 nm.

lumina as well as the spindle cells comprising tumor bundles have essentially the same phenotype (EN-4$^+$, PAL-E$^-$, anti-vWF$^-$, OKM5$^{-/+}$ [40–42]) and similar ultrastructural features (lack of Weibel-Palade bodies, formation of a discontinuous basal lamina, absence of pericytes [43]) as lymphatic endothelial cells [44]. These findings lead us to the conclusion that the neoplastic vascular cells and the spindle cells represent distinct differentiation stages of the same cell type that we will refer to as KS cell. It remains to be seen whether the KS cell is a true neoplasm of mature lymphatic endothelial cells (as one may predict on the basis of its phenotype) or, less likely, originates from any other member of the endothelial cell family.

Ultrastructural studies revealed another major feature of Greek KS, i.e., the presence of TRS and CCC within the cytoplasm of the proliferating KS cells. So far, TRS and CCC have been described as prominent ultrastructural features in the context of AIDS-KS [45–47]. This phenomenon is apparently independent of KS per se in that these structures occur in a wide variety of cells of HIV-1–infected persons, and their presence within peripheral mononuclear cells was even considered as a diagnostic and prognostic marker of AIDS [48–50]. However, the occurrence of TRS and CCC is not pathognomonic for HIV-1 infection. It is actually a not uncommon sequela of certain viral infections and/or virus-induced neoplasms in different species (reviewed in [51]). The more recent demonstration that these structures can be induced in vitro by interferon-α/β [50–52] supports this assumption and therefore suggests that an underlying viral infection is also responsible for the occurrence of TRS/CCC in our patients.

Indeed, extensive electronmicroscopic sampling of KS lesions revealed the presence of rounded 110–130 nm particles exhibiting an electron-dense central core surrounded by an enveloping unit membrane; in addition, we found a particle of similar size with a ring-shaped electron-dense nucleoid and an enveloping membrane with surface spikes. Thus, these particles fulfilled the established morphologic criteria for both mature and immature retroviruses [36–38]. The assumption that the particles disclosed in our study are true virions infecting KS cells rather than some ill-defined "look alike" structures is further supported, albeit not definitively proved, by the detection of such particles exclusively in close association with KS cells in five of 12 patients and by the unequivocal demonstration of a budding particle in a KS cell in one patient.

We are not aware of reports documenting the in situ occurrence of retroviral particles in conjunction with human (tumor) cells—with the notable exception that similar particles have been found budding from KS cells of AIDS patients [53,54]. Although our search for HIV-1, HIV-2, and HTLV-I did not include immunohistologic staining for viral proteins, virus culture, or viral gene amplification by the polymerase chain reaction, our repeated attempts to serologically detect antibodies against proteins encoded by these viruses consistently yielded negative results. This makes it very unlikely that our patients are infected with any of the known human retroviruses. Thus, the possibility should be entertained that the retroviral particles detected in KS lesions from Greek individuals (this study) and, possibly, from AIDS patients [53,54] represent an as yet unidentified human retrovirus.

The crucial question remains whether these newly discovered retroviral particles are of any pathogenetic significance. It is conceivable that they represent exogenous or endogenous non-pathogenic retroviruses occurring exclusively in KS patients. Alternatively, it might even be that extensive ultrastructural tissue screening would reveal their presence in normal skin from healthy individuals.

Should these retroviral particles be involved in KS tumorigenesis, they might trigger events directly resulting in the transformation of endothelial cells. Indeed, others have shown that KS-DNA can transform NIH 3T3 cells [55,56] and that such transformants can induce the appearance of hemorrhagic angiosarcomatous neoplasms in nude mice [56]. On the other hand, the role of retroviruses in KS development could be to induce the production/secretion of angiogenic factors in a given cell as has been reported for CD4$^+$ T cells infected with human retroviruses [57]. Such factors may then act in either a paracrine or autocrine fashion to promote/enhance neoplastic proliferation [57–60]. It remains to be seen whether similar interactions are operative between infiltrating CD4$^+$ T cells and KS cells in our patients.

Our laboratory is currently attempting to rescue and molecularly characterize this putative new retrovirus. The success of these efforts is certainly a conditio sine qua non for further studies to probe a possible biologic role for this retrovirus.

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ANNOUNCEMENT

We are pleased to announce that the 1993 Tricontinental Meeting of The European Society for Dermatologic Research, The Japanese Society for Investigative Dermatology, and The Society for Investigative Dermatology will be held in Japan in the Fall of 1993. We expect that there will be a limited number of grants available to qualified junior investigators, residents, and fellows to help offset travel costs. In addition, the societies are exploring the possibility of obtaining funding to support visiting professorships in Japan at the time of the meeting. Further information will be published in the *Journal of Investigative Dermatology* and can be obtained by contacting The Society for Investigative Dermatology at (216) 844-3682.